the inability of KGF treatment to increase peripheral regulatory T-cell numbers. In summary, pre-transplant administration of KGF can accelerate thymic recovery post allo-HSCT and that the increased export of newly generated T-cells can blunt peripheral expansion of post-thymic T-cells. However, this thymus-dependent effect of KGF is insufficient to further ameliorate cGVHD. Nevertheless, the results suggest a potentially important role of KGF in immune reconstitution and modulation of cGVHD post-allo-HSCT.

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IN VIVO EXPANSION OF CD4+FOXP3+ REGULATORY T CELLS MAY CONTRIBUTE TO CONTROL OF ACUTE GVHD AFTER HLA-MISMATCHED ALLOANERGIZED HSCT

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Many strategies have been explored to selectively remove alloreactive donor T cells to prevent Graft-versus-Host Disease (GvHD) without impairing immune reconstitution after hematopoietic stem cell transplantation (HSCT). An alternative approach is allostimulation of donor T cells with costimulatory blockade (CSB) rendering allospecific cells anergized (hyporesponsive to subsequent alloantigenic challenge). Murine and human data suggest that induction of alloanergy involves cell-mediated suppression, requiring the presence of CD4+CD25+ regulatory T cells (Tregs). We conducted a pilot study of haploidentical alloanergized HSCT after CSB, and measured reconstitution of Treg by intracellular flow cytometry. 5 patients (pts; 4 acute leukemia, one marrow failure) underwent cyclophosphamide/TBI-conditioned haploidentical HSCT with cyclosporine and methotrexate GvHD prophylaxis. Donor bone marrow was incubated with irradiated recipient peripheral blood mononuclear cells and anti-B7.1/2 antibodies for 48 hours to induce alloanergy, washed and infused. All pts engrafted with very rapid reconstitution of T cell subsets, NK cells and immunoglobulins. All evaluable patients had a marked relative increase in peripheral blood CD4+FOXP3+ cells at D + 20–60. CD4+FOXP3+ cells had a memory Treg phenotype (CD25+ CD45RO+CTLA4+ CD127Lo) and were predominantly HLA DR- differentiating them from activated T cells. Despite receiving high doses of donor T cells (median 1.8 (CD4) and 3.1 (CD8) $\times$ 10^7/kg) and achieving full donor chimerism, only 2 pts developed acute GvHD, both Grade II, resolving after short courses of corticosteroids. All evaluable patients also had an increase in CD4+ T effector (Teff) cells with an activated phenotype (CD25+HLADR+FOXP3-) at D + 30–50. Although the antigenic specificity of Teff was not determined, cytokine secretion may have led to reversal of anergy and expansion of alloreactive Teff cells. The marked in vivo expansion of Treg may represent one mechanism of suppressing alloreactive Teff and achieving immunological control of acute GvHD without impairing immune reconstitution in pts receiving HLA-mismatched alloanergized donor T cells. We are using a modification of this strategy in a clinical trial of delayed infusion of escalating doses of alloanergized donor T cells after CD34-selected haploidentical HSCT, to determine the optimal dose of alloanergized donor T cells that abrogates acute GvHD without impairment of immune reconstitution.

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ADMINISTRATION OF rhIL-7 INCREASES TCR REPERTOIRE DIVERSITY THROUGH PREFERENTIAL EXPANSION OF NAIVE T CELLS

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Interleukin-7 (IL-7) is a multifunctional cytokine with critical and non-redundant roles in thymopoiesis and peripheral T-cell homeostasis. We previously reported preliminary results of the first Phase I study of recombinant human IL-7 (rhIL-7), demonstrating that two weeks of alternate day treatment with rhIL-7 produced a marked increase in the number of CD4+ and CD8+ T cells. This increase was maintained in follow up assays at 6 to 12 weeks post treatment. Furthermore, rhIL-7 therapy disproportionately increased CD27+CD45RA+ naive cells, which represent the most diverse elements of the mature T cell receptor (TCR) repertoire, at the expense of CD27-CD45RA+/- effector populations, which are often oligoclonal. In CD8 T cells, the proportion of naive cells increased by 8–39% of total cells. Because of the extent of this population shift, we hypothesized that rhIL-7 treatment would lead to an overall increase in TCR repertoire diversity in CD4+ and CD8+ T-cells. We assessed TCR diversity using spectratype analysis on sorted CD4 and CD8 populations before and one week after rhIL-7 therapy (day 21) in six subjects. For each patient, we determined the divergence of spectratypes in 22 BV families from Gaussian-like normal donor standards and then compared the global diversity of pre and post spectratypes by Wilcoxon paired non-parametric analysis. We determined that rhIL-7 therapy induced a statistically significant increase ($P < .05$) in repertoire diversity in either the CD4+, CD8+, or both T-cell populations in 4 of the 6 patients. This enhancement in diversity was particularly remarkable in that three of these donors were over 60 years of age, and a fourth patient had reduced lymphocyte populations due to recent chemotherapy. Given the short duration of therapy, the age of the patients and the very modest change in TREC we observed, we believe this enhancement in diversity was due primarily to differential population expansion, not IL-7 induced thymic output. Consistent with this interpretation, we observed that a higher percentage of naive T cells than effector T cells remained in cycle (Ki-67+) and maintained elevated levels of anti-apoptotic Bcl-2 during IL-7 therapy. We therefore propose that rhIL-7 has the potential to induce T-cell growth and enhance repertoire diversity, even in lympho-depleted patients with limited thymopoietic capacities, by expanding naive T cell populations.

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THE $CD4^+CD25^+FOXP3^+$ COMPARTMENT FOLLOWING CONDITIONING AND TRANSPLANT: HOST TREG CELLS EXPAND AND COMPRISE THE PREDOMINANT COMPONENT FOR SEVERAL MONTHS DURING RECONSTITUTION POST-HCT

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The capacity of $CD4^+CD25^+Foxp3^+$ (Treg) cells to regulate adaptive and innate immune responses has led to studies investigating their use in novel strategies to regulate allogeneic T cell responses during hematopoietic stem cell transplants (HCT). A fundamental clinical concern post-HCT is the reconstitution of the lymphoid compartment, particularly T cells which can be exceptionally delayed. We have previously found that host Treg cells can regulate resistance to engraftment following HCT, demonstrating that such cells survive and function at least transiently in recipients. The present studies investigated the residual host Treg compartment following varying levels of conditioning (3.0 – 14Gy TBI), and transplant. We found that recipient $CD4^+CD25^+Foxp3^+$ cells: 1) can survive ablative as well as reduced intensity conditioning, 2) undergo expansion (BrdU uptake/cell numbers) and 3) contribute greatly to the Treg compartment for several months post-HCT during which time donor derived Treg cells gradually arise and cede this compartment. Within the first 3 weeks post-lethal conditioning and HCT, 95% of the splenic $CD4^+FoxP3^+$ cells are positive for BrdU, vs. ~40% in normal mice. Using Thy1.1 congenic mice, the vast majority of these cells were found to be resistant (host) Tregs. Two months post-HCT, almost 30% of the compartment was still of host origin. To assess the functional capacity of the residual Treg cell compartment, we examined development of autoimmune disease following transplant of IL-$2R\beta^{-/-}$ BM into syngeneic recipients. Autoimmune disease was prevented in B6-wt but not T cell deficient recipients. Interestingly, the failure to transfer autoimmune disease following IL-$2R\beta^{-/-}$ HCT into B6-$CD4^{-/-}$ recipients was associated with the presence of a peripheral $CD8^+FoxP3^+$ population not detected in B6-wt mice. This finding